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STUDIES ON THE pH-DEPENDENCE, INHIBITION, AND REACTIVATION OF ANGIOTENSIN II AND ANGIOTENSIN II AMIDE-CLEAVING ENZYMES OF HUMAN PLASMA

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STUDIES ON THE pH-DEPENDENCE, INHIBITION, AND REACTIVATION OF ANGIOTENSIN II- AND ANGIOTENSIN II AMIDE-CLEAVING ENZYMES OF HUMAN PLASMA

by

M. Zwanzig and W. Oelkers*

We compared the pH-dependence of the biological inactivation of α-L-aspartyl[1]-angiotensin II (A) and of α-L-asparaginyl[1]-angiotensin II (H) by human plasma. In addition, the pH-dependence of the EDTA inhibition of "angiotensinases" and its reactivation by divalent metal ions were investigated. Both substrates are cleaved by at least three enzymes with different pH optima. Distinguishable chracteristics of the A- and H-inactivating enzymes were found in all pH ranges. We also compared the pH-dependence of the biological inactivation of H and the cleavage of asparagine from H (aminopeptidase). Our results are compatible with the assumption that the enzymes most active at pH 5.6 are endopeptidases and that the enzymes most active in the neutral and alkaline domains are aminopeptidases.

Regoli et al. [1] were the first to suggest that asparaginyl[1]-angiotensin II (H) and aspartyl[1]-angiotensin II (A) are inactivated by different enzymes in blood plasma. Nagatsu et al. [2] were able to substantiate this assumption to a great extent. They found that the hydrolysis of α-L-aspartyl[1]- and α-L-glutamyl-β-naphthylamide are competitively inhibited by A, but not by H. The hydrolysis of L-leucyl-β-naphthylamide, on the other hand, is competitively inhibited only by H. Whereas hydrolysis of the N-terminal peptide bond of A in undiluted serum at pH 7.0 is activated by 12 mM Ca^{++} and completely inhibited by 2.4 mM Ni^{++}, the corresponding reaction for the hydrolysis of H is inhibited weakly by Ca^{++} and only by 50% by Ni^{++}. Similar differences were

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found in studies of the hydrolysis of α-L-aspartyl-β-naphthylamide and L-leucyl-β-naphthylamide in the presence of Ca^{++} and Ni^{++} [3]. The authors emphasized the similarity of the inhibiting and activating characteristics of the enzymatic degradation of A in plasma with those of an enzyme (aminopeptidase A), found by Glenner and McMillan [4] in rat kidneys, which specifically cleaves the N-terminal α-L-aspartyl- and α-L-glutamyl-peptide bonds. For the hydrolysis of H, on the other hand, several less specific peptidases are assumed, some of which also cleave the N-terminal L-leucyl-peptide bonds.

The "angiotensinase A" of human plasma, described by Khairallah et al. [5] supposedly cleaves N-terminal α-L-aspartyl- and α-L-asparaginyl-peptide bonds specifically. Angiotensin analogs such as β-aspartyl^1-angiotensin II and arginyl^1-angiotensin II are not attacked. Later, however, Khairallah et al. [6] were able to separate the α-L-aspartyl-peptide-cleaving enzyme activity from the α-L-asparaginyl-peptide-cleaving enzyme activity by ammonium sulfate fractionation. The partially contradictory data in the literature on substrate specificity, inhibition, and reactivation of angiotensin-cleaving enzymes in plasma are gradually beginning to be untangled.

In the immediately preceding paper [7], we have already indicated that the significance of plasma angiotensinases (ATases) for limiting the biological activity of angiotensin is probably slight. What is important, however, is a knowledge of their properties in in vitro experiments, e.g. in the determination of renin. The ATases have to be inactivated in order that the angiotensin formed in the enzymatic reaction between renin and angiotensinogen (renin substrate) not be destroyed and that it be quantitatively determinable. Since for some methods of renin determination the renin-substrate reaction does not occur in the neutral range [8-10], knowledge of the angiotensin-cleaving enzymes active in the weakly acidic range is also important.

In the following, we report on comparative investigations of the biological inactivation of A and H as a function of the hydrogen ion concentration in human plasma. In addition, we investigated the hydrogen ion concentration-dependence of the inactivation of ATases by the chelate-forming ethylenediamine tetra-acetic acid (EDTA), and their reactivation by divalent metal ions. Since a comparison of the substrates A and H appeared important to us, we were not able to use the optical tests described by us [11] for determining the angiotensin-aminopeptidase activity. The large quantities of aspartyl^1-angiotensin II required for this test were not available. We therefore employed a procedure used in many previous investigations [1, 5, 6, 12]. The loss of vasopressor action of a known quantity of angiotensin after incubation with plasma under defined conditions is measured in the narcotized rat. Only relative enzyme activities can be determined by this method, since no substrate saturation of the enzyme is obtained and since the reaction rate constantly diminishes during the incubation due to the decrease in substrate concentration. The method satisfies the concern of this investigation, however, and is adequately accurate.

Experimental Method

Experimental Material

The experiments were carried out with heparin plasma from healthy human subjects (2000 u. heparin/100 ml whole blood). The plasma was stored at -20° until further use.

Incubation of Plasma With Angiotensin for Determination of its Biological Inactivation

The incubation batches contained:

1.0 ml substrate solution [500 ng α-L-aspartyl^1-angiotensin II or 500 ng α-L-asparaginyl^1-angiotensin II (Hypertensin-CIBA), dissolved in 1 ml 0.05 M

tris-maleate buffer of different pH values].

3.9 ml 0.05 M tris-maleate buffer of the same pH value. The tris-maleate buffer was prepared according to the data in the Scientific Tables, Documenta Geigy 1962, pp. 277-278. For the determination of the pH-dependence of the biological inactivation of A and H, intervals of 0.2-0.4 pH units were chosen between pH 5.2 and pH 8.6. For the reactivation experiments with different metal ion concentrations, three pH values were chosen in turn, corresponding to the previously ascertained pH optima for biological inactivation.

0.5 ml 0.9% NaCl solution for the determination of the pH optimum curves for both substrates. For incubation with EDTA or metal salts, 10 times the desired final concentration of Na_2-EDTA (Titriplex III, Merck) or of the metal dichloride, or of both together in 0.5 ml 0.9% NaCl solution, was added. Prior to this, the solutions were adjusted to pH 7.0 with NaOH.

0.1 ml of a 5% aqueous solution of neomycin sulfate (Bykomycin, BYK-Gulden) to prevent bacterial growth.

0.5 ml mixed plasma, with pH adjusted to 7.0.

The batches were prepared in an ice bath. Immediately after addition of the plasma and thorough mixing, 2 ml were removed as a blank value (non-incubated batch) and heated for 10 min in closed glass test tubes in a water bath at 100°. The coagulated proteins were centrifuged off at 3000 rpm and the supernatant was frozen at -20° in polystyrene test tubes for subsequent testing in rats. The remaining batch (3 ml) was incubated at 37° for 10 min when the substrate was H, and 120 min when the substrate was A. After the incubation, the batches were immediately heated to 100° and treated further like the blanks.

After determination of the pH optima of angiotensin inactivation by human plasma, the inhibition of the ATases was tested by dialysis against 0.9% NaCl solution or against Na_2-EDTA in 0.9% NaCl solution.

50 ml of plasma were each: a) Immediately frozen; b) Dialyzed for 9 days at 3° against 0.9% NaCl solution; c) Dialyzed for 5 days at 3° against 10 mM or 1.0 mM Na_2-EDTA in 0.9% NaCl solution (adjusted to pH 7.0), and then for 4 days against 0.9% NaCl solution. The dialyzate was changed daily. After the dialysis, volume differences in the plasma samples were evened out by the addition of 0.9% NaCl solution.

The biological inactivation of A and H by untreated or dialyzed plasma was then tested (for incubation batch, see above). A was incubated with plasma at pH 5.6, 6.8, and 8.0, and H at pH 5.6, 7.2, and 8.6. The incubation times were chosen so that for each pH value, about 50% of substrates A or H was inactivated by untreated plasma (see Table 1). Then the reactivation of the ATases inhibited by dialysis against EDTA was tested. The batches contained varying concentrations of $CaCl_2$, $MgCl_2$, $ZnCl_2$, $MnCl_2$, and $CoCl_2$.

Angiotensin Determination in Rats

Male Wistar rats (200-300 g) were nephrectomized under pentobarbital narcosis. After 24 h, they were tracheotomized under urethane narcosis (2 ml 20% urethane solution i.m.). Polyethylene catheters were inserted in the right jugular vein and in the left carotid artery. Pressure in the carotid artery was recorded via a Hg manometer with an ink pen on a kymograph. 0.2 ml of the solution to be tested, or 0.2 ml of the angiotensin standard solution, was injected in the jugular vein. 0.1 ml Ringer solution was then injected. The angiotensin standard solutions contained 50 ng or 100 ng of Hypertensin-CIBA in 1 ml Ringer solution. Each unknown sample and the blanks also were compared twice with the immediately previously and subsequently injected Hypertensin standard. All inactivation rates given are mean values of two determinations. The biological inactivation is given as the percent loss in pressor activity of the incubated batch compared with the non-incubated batch. In batches which

Table 1. Biological inactivation of A and H at various pH values after dialysis against 0.9% NaCl solution or against 10 mM Na_2-EDTA solution. Reactivation of angiotensin-degrading enzyme after dialysis against Na_2-EDTA, by divalent cations at various concentrations (given as final concentration in 1:10 diluted plasma). Numbers: Percent loss in pressor activity. — partial inhibition. – – total inhibition. + partial reactivation. ++ total reactivation. O no reactivation.

	Substrate	α-L-Aspartyl¹-Angiotensin II (A)			α-L-Asparaginyl¹-Angiotensin II (H)		
	pH	5,6	6,8	8,0	5,6	7,2	8,6
a	Inkubationszeit (Min).	720	180	180	60	15	15
b	Plasma 1:10 verdünnt	47	48	50	39	53	45
c	Nach Dialyse gegen 0,9proz. NaCl	0 —	21 —	25 —	0 —	25 —	23 —
d	Nach Dialyse gegen 0,01M Na_2EDTA	0 —	0 —	0 —	0 —	15 —	12 —
e	Nach anschließender Reaktivierung mit						
	0,25 mM Ca^{++}	22 +	44 ++	50 ++	0 O	58 ++	50 ++
	2,5 mM Ca^{++}	47 ++	45 ++	50 ++	0 O	56 ++	50 ++
	0,1 mM Mg^{++}	1 O	0 O	44 ++	0 O	53 ++	0 O
	1 mM Mg^{++}	0 O	5 O	45 ++	0 O	50 ++	5 O
	5 μM Zn^{++}	35 +	5 O	36 +	/	40 +	24 (+)
	50 μM Zn^{++}	45 ++	6 O	45 ++	33 ++	41 +	45 ++
	500 μM Zn^{++}	38 +	5 O	33 +	38 ++	25 +	0 O
	10 μM Mn^{++}	0 O	7 O	9 O	0 O	41 +	0 O
	100 μM Mn^{++}	5 O	7 O	15 (O)	0 O	50 ++	0 O
	1 μM Co^{++}	7 O	30 +	33 +	0 O	46 ++	0 O
	100 μM Co^{++}	11 (O)	47 ++	50 ++	4 O	46 ++	5 O

a. Incubation time (min)
b. Plasma, diluted 1:10
c. After dialysis against 0.9% NaCl
d. After dialysis against 0.01 M Na_2-EDTA
e. After immediately subsequent reactivation with:

contained 0.9% NaCl solution instead of plasma, spontaneous hydrolysis of angiotensin was not found for any of the pH values tested between 5.2 and 8.6.

Angiotensin determination by the procedure described is well reproducible. For 31 arbitrarily chosen double determinations, the differences in inactivation rate for two determinations with the same sample on two different rats were, on the average, 3.9% of the mean value of both determinations (0% to 10%). The

standard deviation was 3.1%. Inactivation rates above 10%, or differences between different inactivation rates greater than 10%, should therefore be considered significant.

Measurement of Asparagine Cleavage from α-L-asparaginyl[1]-angiotensin II Aminopeptidase Activity

The principle of the method developed by us was indicated in the immediately preceding paper [7] and is described in greater detail in [11]. To compare the pH-dependence of the aminopeptidase activity of plasma with respect to H with the pH-dependence of the biological inactivation of H, incubation was carried out for 60 min in 0.05 M tris-maleate buffer, pH 5.2-8.6, instead of in 0.067 M sodium phosphate buffer [11]. The aminopeptidase activity (Fig. 2) is given here not in i.u./l, but rather in extinction units.

Results

Comparison of pH-Dependence of Biological Inactivation of A and H by Human Plasma (Fig. 1)

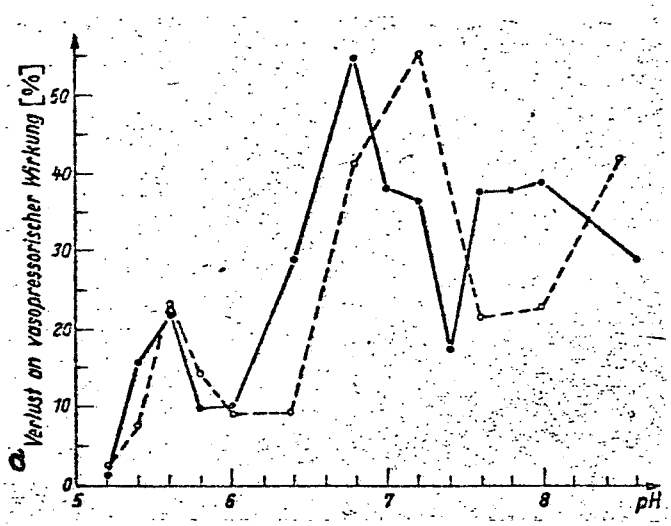

Fig. 1. Comparison of biological inactivation of A (•—•) and H (o---o) by 1:10 diluted plasma as a function of pH of 0.04 M tris-maleate buffer. Incubation time with substrate H, 10 min; with substrate A, 120 min. Each point corresponds to the mean value of two incubation series.

a. Loss of pressor activity

For comparable inactivation, A must be incubated about 10 times longer with the diluted plasma than H. The inactivation optima of A are at pH 5.6, 6.8, and 7.6-8.0. The maximum inactivation is at pH 6.8. The inactivation optima of H are at pH 5.6 and 7.2. A further increase in inactivation of H is observed beyond pH 8.0, whose maximum could not be ascertained in tris-maleate buffer. The pH optima for the inactivation of A and H thus agree in the acidic range, whereas differences are found in the neutral and alkaline ranges.

Comparison of pH-Dependence of Biological Inactivation of H With the Cleavage of H by Aminopeptidases (Fig. 2)

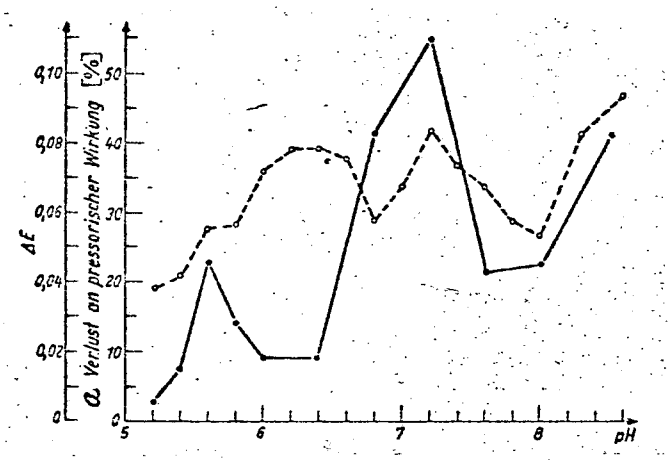

Fig. 2. Comparison of biological inactivation (% loss of pressor activity) of H (•—•) with rate of asparagine cleavage from H (o---o), as a function of pH of 0.04 M tris-maleate buffer. The aminopeptidase activity (mean value of two incubation series) is given in extinction units (366 nm).

a. Loss of pressor activity

In agreement with previous results for the determination of the pH optimum in acetate and phosphate buffers [11], we found a pH optimum for aminopeptidase activity with respect to H at pH 6.2-6.4. A further optimum, not sharp compared with the previous one, was found at pH 7.4, in agreement with the optimum for the biological inactivation of H. Beyond pH 8.0, the

aminopeptidase activity with respect to H again increases, similarly to biological inactivation.

The Effect of Ca^{++} and Na_2-EDTA on the Biological Inactivation of A and H, as a Function of pH (Fig. 3 and 4)

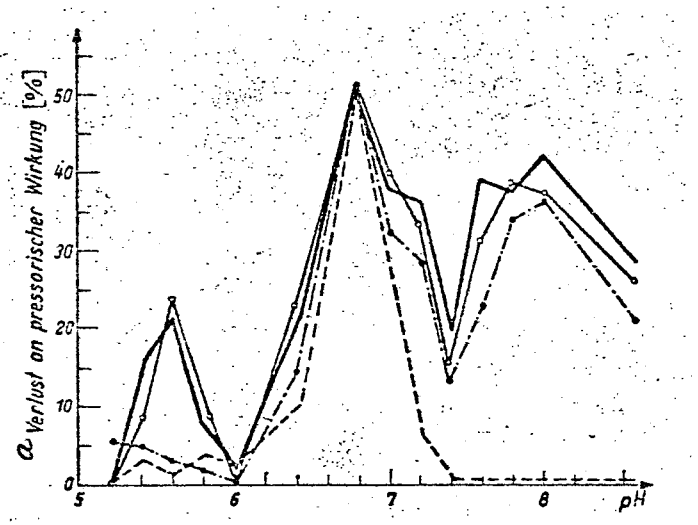

Fig. 3. Biological Inactivation of A by 1:10 diluted human plasma, as a function of pH of 0.04 M tris-maleate buffer: Without addition of affectors (——); with 1 mM Na_2-EDTA (---); with 1 mM $CaCl_2$ (o—o); with 1 mM Na_2-EDTA + 3 mM $CaCl_2$ (•-•-•-•). Results of one incubation series each. Incubation time: 120 min.

a. Loss of pressor activity

Na_2-EDTA (1 mM) inhibits the biological inactivation of A and H almost completely in the acidic range (pH optimum: 5.6). Calcium, which does not significantly alter the degradation of A and H when added alone in 1 mM concentration, does not counteract the EDTA-inhibition upon addition of a two-fold excess (3 mM Ca^{++}). The trivial inactivation of A and H by about 5% at pH 5.6 in the presence of Ca^{++} and EDTA is insignificant. In the neutral range, 1 mM EDTA does not significantly inhibit the biological inactivation of A and H. Ca^{++} and EDTA + Ca^{++} also do not significantly influence the inactivation of A and H.

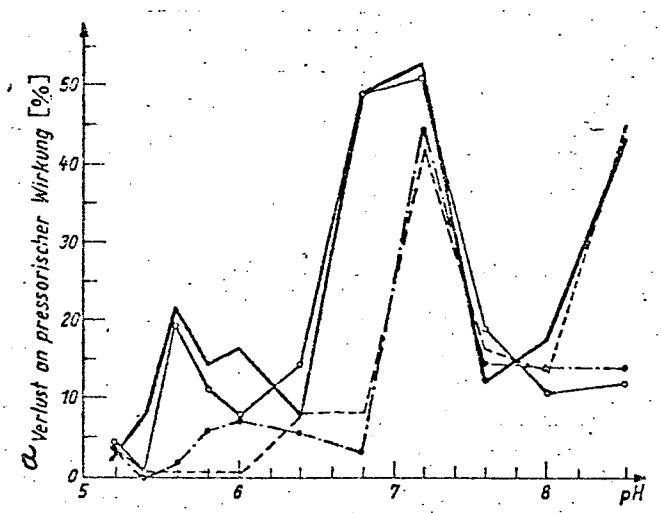

Fig. 4. Biological inactivation of H by 1:10 diluted human plasma with and without affectors. For curve designations, see Fig. 3. Incubation time: 10 min.

a. Loss of pressor activity

Clear differences between A and H are found in the alkaline range. EDTA (1 mM) completely inhibits the inactivation of A. Ca^{++} (inactive at 1 mM concentration) completely counteracts the EDTA-inhibition at 3 mM concentration. The biological inactivation of H is partially inhibited by EDTA only from pH 8.0 on up. Ca^{++} does not counteract the EDTA-inhibition.

Inhibition of Biological Inactivation of A and H by Dialysis of Plasma Against 0.9% NaCl Solution and EDTA Solution, and Reactivation by Divalent Metal Ions (Table 1)

Dialysis of plasma against 0.9% NaCl solution completely inhibits the biological inactivation of A and H in the acidic range. In the neutral and alkaline ranges, the biological inactivation of A and H is partially inhibited.

10 mM EDTA completely inhibits the biological inactivation of A in all pH ranges. The inactivation of H is completely inhibited in the acidic range, but less than completely in the neutral and alkaline ranges.

Ca^{++} (2.5 mM) counteracts the inhibition of the biological inactivation of A by EDTA dialysis in all pH ranges. 0.25 mM Ca^{++} reactivates completely at

ph 6.8 and 8.0, and partially at pH 5.6. By contrast, Ca^{++} has no effect on the inhibition of the biological inactivation of A by EDTA dialysis at pH 5.6, whereas 0.25 mM Ca^{++} reactivates completely at pH 7.2 and 8.6.

Mg^{++} (0.1 mM) reactivates the biological inactivation of A only at pH 8.0, and of H only at pH 7.2. No reactivation occurs in any other pH range.

Zn^{++} reactivates the biological inactivation of A at pH 5.6 and 8.0 already at very small concentrations (5-50 µM), whereas no reactivation occurs at pH 6.8. Higher Zn^{++} concentrations (0.5 mM) reactivate only partially at pH 5.6 and 8.0. An inhibition of the reactivated enzyme probably already occurs here; we were also able to observe this with the use of even higher Zn^{++} concentrations in preliminary experiments.

Mn^{++} (10-100 µM) completely reactivates only the biological inactivation of H at pH 7.2. The reactivation of the degradation of A at pH 8.0 (15% inactivation) can not be considered certain, since the error in the angiotensin determination in rats with Mn^{++}-containing solutions was somewhat larger than in the testing of other metal ion-containing solutions, which did not cause any changes in the accuracy of the biological test.

Co^{++}, in concentrations of 1-100 µM, completely reactivates the biological inactivation of A at pH 6.8 and 8.0, and of H at pH 7.2. In other pH ranges, Co^{++} is ineffective.

Triple dialysis of plasma against 1 mM EDTA completely inhibits the biological inactivation of A and H at pH 5.6. In the other pH ranges, only partial inhibition occurs. As would be expected, reactivation experiments with divalent cations gave the same results as were obtained after dialysis of the plasma against 10 mM EDTA solution. Of course, the accuracy of the reactivation experiments is lowered due to the only partial inhibition of the angiotensinases in the neutral and alkaline ranges. The results are therefore not presented in detail.

Discussion

The results show that both A and H are inactivated by at least three enzymes of human plasma with different pH optima. The actual activity maximum is in the neutral range, although the pH optima of the A- and H-cleaving enzymes differ from each other by 0.4 pH units. The pH optima for the biological inactivation of A and H in the acidic range are identical. There are again differences in the weakly alkaline range. The pH optimum for the inactivation of A is at pH 7.6-8.0, and that of H is further into the alkaline range. The pH optima for the release of asparagine from H during substrate saturation are at pH 6.2-6.4 and 7.2. Only the activity maximum at pH 7.2 and the renewed increase in asparagine cleavage in the alkaline range correspond to the pH dependence of the biological inactivation of H. The maximum for asparagine cleavage at pH 6.2-6.4 could correspond to an enzyme with low substrate affinity, which for substantially lower substrate concentrations, such as are used for the measurement of biological inactivation, converts only a small amount of substrate. The pH optimum for biological inactivation observed at pH 5.6, on the other hand, is missing in the pH optimum curve for aminopeptidase activity. This result could support the supposition of Khairallah et al. [6] that the "acidic angiotensinase," about which Pickens et al. [10] had already reported, is an endopeptidase. Pickens et al. [10] and Khairallah et al. [6] found that the angiotensinase activity in this range can be inhibited with di-isopropyl-fluorophosphate. They concluded from this that the mode of action is similar to that of chymotrypsin. Khairallah et al. [6] have recently been able to separate the "acidic ATase" from angiotensinase A by ammonium sulfate fractionation and chromatography over Sephadex G-75. They found the optimum activity between pH 5.8 and 6.0. The enzyme cleaves both A and H, the latter twice as fast as the former. But even angiotensin analogs such as β-aspartyl^1-angiotensin II and succinyl^1-angiotensin II, which

are not attacked by angiotensinase A, can inactivate the enzyme. Khairallah et al. [6] found that the enzyme is inactivated by dialysis against EDTA and is not reactivated by Ca^{++}, Mn^{++}, Mg^{++}, or Co^{++}. They concluded that the inactivation by EDTA was determined not by chelate formation with a metallic cofactor, but rather by denaturation of the enzyme. Apparently, Khairallah et al. [6] carried out their reactivation experiments only with the substrate H. Their results then agree completely with our own (See Table 1). Zn^{++}, which Khairallah et al. did not test as a potential reactivator, is nevertheless able to completely counteract the EDTA inhibition already at very low concentrations (Table 1). The inhibition of the degradation of A by EDTA at pH 5.6, on the other hand, is counteracted not only by Zn^{++} but also by Ca^{++}. Since the stability constant of the EDTA-calcium-chelate at pH 5.6 is substantially smaller than in the neutral range [13], and since the "acidic ATase" is already inhibited by dialysis against 0.9% NaCl solution or by the addition of small EDTA concentrations to the reaction mixture (Fig. 3 and 4), it is unlikely that Ca^{++} is the natural cofactor of the A-cleaving "acidic ATase." The stability of the EDTA-zinc-chelate is about five orders of magnitude greater than that of the EDTA-calcium chelate [13]. It is therefore probable that Zn^{++} or a metal ion with a similar affinity for EDTA is the natural activator of "acidic ATase." The inability to inactivate the enzyme by dialysis against 0.9% NaCl solution is also evidence against a strong bond between this metal ion and the enzyme protein. It can be concluded from the activation of the A-cleaving enzyme activity by Ca^{++} that the "acidic ATase" consists of two enzymes, which on the one hand inactivate H (and A?), and on the other hand inactivate only A.

For the "neutral ATases," there is substantial agreement between the results of Khairallah et al. [6] and ours. Khairallah et al. took the angiotensinase A, which was separated from the "acidic ATase" (angiotensinase B) by ammonium sul-

fate fractionation [5,6], and were able to separate it into two enzymes by further fractionation. Of these enzymes, one (angiotensinase A1) inactivates only H, whereas angiotensinase A2, which precipitates at 2.35 M $(NH_4)_2SO_4$-concentration, inactivates only A. Both are completely inhibited by dialysis against EDTA (3 mM), and reactivated by Ca^{++}. The pH optimum for ATase A1 is at pH 7.4, and that of ATase A2 is at 6.8. The different pH optima for the inactivation of A and H in the neutral range, which we found, are thus not due to lack of experimental precision, as one could have suspected, but rather indicate the existence of two enzymes. Differences are also found in the reactivation with divalent cations of the enzymes which are inhibited only by higher concentrations of EDTA (Fig. 3 and 4, Table 1). Whereas the EDTA-inhibited cleavage of A at pH 6.8 is reactivated only by Ca^{++} and Co^{++}, that of H at pH 7.2 is reactivated by Ca^{++}, Mg^{++}, Mn^{++}, and Co^{++}. Here, moreover, differences are obtained compared to the results of Khairallah et al. [6], who found total reactivation of the EDTA-inhibited complex angiotensinase A only with 1 mM Ca^{++}, and partial reactivation with 1 mM Mn^{++} and Co^{++}. 1 mM Mg^{++} was inactive. Of course, they did not indicate which substrate (A or H) they used here. Some of the differences between our results and those of Khairallah et al. [6] could, however, be explained by the different concentrations of metal ions. In preliminary experiments, we found that already 0.5 mM Mn^{++} or Co^{++} can partially inhibit the neutral ATases, whereas lower concentrations completely reactivate them. If only one metal ion concentration is being tested, this partial inhibition can also be interpreted as partial reactivation.

The enzymes which inactivate A and H in the weakly alkaline range differ sharply with respect to their pH-dependence and reactivation by metal ions after dialysis against EDTA. The H-inactivating enzyme activity above pH 8.0 is not inhibited until higher EDTA concentrations than the A-cleaving enzyme (pH optimum:

7.6-8.0) (Figs. 3 and 4, Table 1). Reactivation by Ca^{++} and Zn^{++} occurs, whereas the A-cleaving enzyme is completely reactivated by Ca^{++}, Mg^{++}, Zn^{++}, and Co^{++}. In this range also, our investigations cannot exclude the participation of several enzymes in the inactivation of the respective substrates. Comparison of the pH-dependence of the biological inactivation of H and the asparagine cleavage from H (Fig. 2) allows one to conclude that the "neutral" and the "alkaline" H-cleaving ATases are aminopeptidases.

Our results and those of Khairallah et al. [6] are corroborative in many respects. To some extent, contradictions can be explained by the different experimental conditions. The heterogeneity of the "angiotensinases" is remarkable. In future investigations, it should be taken more into account.

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